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US APPLICATION NO
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INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/13340	June 29, 1999	June 11, 1998

TITLE OF INVENTION
CONTROL OF FLOW AND MATERIALS FOR MICRO DEVICES

APPLICANT(S) FOR DO/EO/US
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Applicant herewith submits to the United States Designated /Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(I).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:
    copy of published PCT application WO 99/64851

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED	09/701662
PCT/US99/13340	June 11, 1999	June 11, 1998	

17. [ ] The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)-(5):

Neither international preliminary examination fee (37 CFR 1.482)

Nor international search fee (37 CFR 1 445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO (1.492(a)(3)) $1,000.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO (1.492(a)(5) $860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO(1.492(a)(2)) $710.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) (1.492(a)(1)) $690.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ..... $ 100.00

CALCULATIONS PTO USE ONLY

ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than **[ ] 20 [ ] 30** months from the earliest claimed priority date (37 C.F.R. 1.492(e)).	$130.00	

Claims	**Number Filed**	**Number Extra**	**Rate**	$	
Total Claims	17-20=	0	X $ 18.00	$0	
Independent Claims	3-3=	0	X $ 80.00	$0	
Multiple dependent claim(s) (if applicable)			+ $270.00	$0	

TOTAL OF ABOVE CALCULATIONS =	$990.00	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).	$495.00	
SUBTOTAL =	$495.00	
Processing fee of $130.00 for furnishing the English translation later than **[ ] 20 [ ] 30** months from the earliest claimed priority date (37 CFR 1.492(f)). +	$	
TOTAL NATIONAL FEE =	$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +	$	
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a. [X] A check in the amount of $ 495.00 to cover the above fees is enclosed.

b. [ ] Please charge our Deposit Account No. 02-4377 in amount of $___ to cover the above fees. A copy of this sheet is enclosed.

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NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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CONTROL OF FLOW AND MATERIALS FOR MICRO DEVICES

INTRODUCTION

The present invention generally relates to methods and devices for the control of the movement of fluids and electrically charged sample components within those fluids. More particularly, the present invention permits exclusion or concentration of specifically chosen sample components within a fluid.

The present invention provides an analytical device, either microchip- or capillary-based, having the means to exclude specific sample components of interest from a capillary or channel for the purpose of preconcentration or control of movement of sample components. Such a control system includes a means for controlling the flow of the fluid in the channel and the placement of an electrode at the immediate entrance of each channel on such devices so that material may be directly manipulated by either or both of the effects of both bulk flow and electrically driven migration.

BACKGROUND OF THE INVENTION

Capillary zone electrophoresis (CZE) is an efficient analytical separation technique which utilizes differences in mobility of sample components in an electric field based on the electrical charge and molecular site and shape of the sample component. Conventional CZE systems typically comprise a buffer-filled capillary with outlet and inlet ends disposed in two reservoirs into which one sample is injected, a means for applying voltage to the capillary resulting in migration of the sample through the capillary, and a means for detecting the sample zone.

Sample injection systems and capillary zone electrophoresis channel systems have been integrated together on planar glass substrates for separation of sample components as described by Harrison et al. (1992, Anal. Chem. 64:19261932) and Seiler et al. (1993, Anal. Cem. 65:1481-1488). Additionally, capillary electrophoresis on microchips has been described by Manz et al., (1992, J. of

Chromatography 593:253-258). Total chemical analysis systems (TAS) in which sample transport, chromatography or electrophoretic separations and detection are all performed have also been developed.

One of the limitations of conventional CZE is the extremely small amount of sample which must be used in order to obtain separation or resolution of sample components. The use of small volume samples results in low amount of sample components of interest representing a major limitation in the detectability of sample components. On the other hand, the larger the sample volume introduced into the capillary, the broader the sample component peaks will be. Attempts to increase injection sample volume typically leads to a breakdown in resolution due to broadening of the peaks attributable to individual sample components which one is actually trying to resolve or separate and possibly leads to generation of laminar flow inside the capillary.

A number of techniques have been developed for increasing the concentration of specific sample components of interest and narrowing the width of the injected sample. One such technique involves the use of a solid-phase adsorption medium followed by a sequential combination of pressure- and electrically-driven flows as described in United States Patent No. 5,453,382. Using such a technique, the solution containing the sample component of interest is applied to the solid phase adsorption medium under conditions which permit sorption of the sample component of interest to the adsorption medium. The environment of the medium is then altered to promote desorption of the concentrated sample component and a voltage gradient is induced across the medium to induce electroosmosis. United States Patent No. 5,340,452 also describes a similar method for increasing the concentration of sample components prior to electrophoresis by using an active material which selectively retains the sample components of interest at the inlet end of the capillary tube.

For some specialized samples, another obstacle to successful separation of components of a solution results from the low strength of the electric field in the buffer bordering the sample solution and the column buffer. To circumvent this problem, water or diluted buffer may be removed from the capillary or column using electro-osmotic flow while the sample components are stacked in a

support buffer thereby concentrating the sample components in a sample with a minimum amount of laminar flow. Such a method is described in United States Patent No. 5,116,471.

For large volume samples in constrained containers, pressurized flow and countermigration can be used to increase the overall concentration as described by Hori et al. (1993, Anal. Chem. 65:2882-2886). The sample is introduced into a first vessel containing buffer which is connected to another vessel by a glass tube. An electrode extending into the first vessel applies a voltage to the sample while suction pressure is applied. The sample concentration increases throughout the first vessel rather than concentrating the sample in a discrete portion of that vessel because the applied potential field is unconstrained throughout the buffer volume. Because the concentration increase and electric fields are dispersed throughout the entire first vessel volume this technique is not applicable as a small volume injection/preconcentration technique. Moreover, this arrangement does not allow for micromanipulations such as electrophoretic separation within the vessel containing the concentrated sample.

Hence, none of the aforedescribed methods provide for concentration of sample components upon immediate introduction into a constrained small volume flow path which receives a fluid sample without the use of complicated systems such as discontinuous buffer systems and, in some instances, microengineered absorption devices. Accordingly, there exists a need in the art for more precise and efficient methods and devices for increasing the concentration of sample components of interest within a fluid sample while maintaining a consistent buffer and without microengineering absorption systems.

SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide a novel, more efficient method for controlling the movement of fluids and electrically charged species, referred to as sample components, within those fluids which permits exclusion or concentration of specifically chosen species within a constrained fluid-flow path.

It is another object of the invention to provide an analytical electrophoretic arrangement including microchips or capillaries which excludes specific sample components of interest from a capillary or channel for the purposes of preconcentration or control of movement of materials.

It is a further object of the present invention to provide an arrangement in which preconcentration and manipulation is achieved within a single constrained flow pathway system. More particularly, the sample is preconcentrated in a portion of the constrained flow pathway and is manipulated as it travels through the pathway.

These and other objects of the invention are obtained by a method for controlling the movement of a specific sample component in a fluid sample comprising:

(a) providing a constrained fluid pathway having an inlet;

(b) introducing the fluid sample into the inlet of the constrained fluid pathway;

(c) providing an electrode mounted at the inlet of the fluid pathway, the electrode being entirely external to the constrained fluid pathway;

(d) applying voltage to the electrode to create a voltage gradient within the constrained fluid pathway to promote electrophoretic migration of the sample component; and

(e) adjusting the flow rate of the fluid approximately equal to and opposite to the electrophoretic migration of the sample.

(f) adjusting the electrophoretic migration rate to be approximately equal and opposite to the flow rate of the fluid.

wherein movement of the specific sample component ceases.

The invention further provides an electrophoretic apparatus for controlling the movement of an sample component in a fluid sample comprising:

(a) at least one constrained fluid pathway having an inlet and an electrode mounted at the inlet of the constrained fluid pathway and entirely external to the constrained fluid pathway; and

(b) a power supply for supplying a voltage to the electrode.

It is another object of the invention to provide an electrophoretic apparatus for controlling the movement of an sample component in a fluid sample comprising:

(a) at least one injection fluid pathway having an electrode mounted at the inlet of said the pathway;

(b) at least one separation fluid pathway having an electrode mounted at the inlet of said pathway;

(c) at least one power supply for providing voltage between the electrodes; and

(d) means for regulating the bulk flow within the channels.

The present invention can be utilized in methods and devices for manipulating, testing, probing, or analyzing sample fluids of any kind where fluid manipulations are utilized for preconcentration, chemical reaction, injection, detection, or movement, or cessation of movement, of components of interest in a sample fluid.

In one embodiment, the present invention is directed to an analytical device having a plurality of channels with electrodes placed at the immediate entrance of all or selected channels and a method for regulating the bulk flow within the channels. When the bulk flow is set approximately equal to and opposite the electrophoretic migration of specific sample components of interest, the movement of those specific sample components ceases. The introduction of an electric field between the electrodes within the channel, coupled with control of bulk flow, allows selected sample components of interest to be excluded or preconcentrated immediately upon introduction of the fluid sample into the channel.

BRIEF DESCRIPTION OF THE DRAWINGS

Further objects and advantages of the invention will be apparant from a reading of the following description in conjunction with the accompanying drawings, in which:

Figure 1 is a schematic drawing of a fused silica capillary arrangement with electrodes placed immediately at the inlet to provide the voltage control within

the capillary in accordance with the invention;

Figures 2(a), 2(b) and 2(c) are schematic drawings of a micro-device apparatus having an injection channel and a separation channel in accordance with the invention;

Figure 3 is a schematic drawing of a micro-device apparatus indicating the preconcentration of materials at the immediate entrance to a channel where the voltage with in the buffer reservoir is held constant in accordance with the invention;

Figure 4 is a schematic drawing of the theoretical profile of the preconcentration of material at the immediate entrance to a capillary showing the concentration of desired materials;

Figure 5 is a graph showing the normalized fluorescence intensity versus distance outside the capillary entrance for two control experiments;

Figure 6 is a graph showing the normalized fluorescence intensity versus number of pixels (1 pixel = 0.24 μm) outside a capillary entrance ;and

Figures 7(a) and (b) are fluorescence micrographs of a capillary entrance before and after, respectively, preconcentration of 200 nm fluorescently labeled latex micro spheres for 270 seconds.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides novel methods and devices for exclusion or concentration of specifically chosen sample components within fluids through the control of fluid movement and electrophoretic migration of charged sample components within those fluids. Typically the fluid sample is delivered or injected into a restricted flow path such as a channel or capillary. For purposes of the present invention, the flow path is preferably less than 200 microns in diameter. Precise control of fluid manipulation, sample component movement and solution injection systems are accomplished by carefully controlling the voltage field gradients and the bulk flow within each channel on a micro-device.

The principle of electrophoretic focusing as a means of sample component exclusion from a capillary or channel can be applied to the microscale analytical device described herein. The apparatus and processes disclosed herein may

be used on microchip instrumentation in conjunction with control fluid dynamics in channels formed into or onto semiconductor devices. As used herein, the term "microchip" includes a semiconductor device comprising silica or any other substrate which may be used in microfluidic devices, which may be used in or in conjunction with a computer.

The present invention also provides for the placement of an electrode at the immediate entrance of each channel on a micro-device so that material movement may be directly manipulated by electrically-driven migration, i.e., electrophoretic migration. The present invention also provides control of bulk flow of the fluid within the channel. Bulk flow may be positive or negative depending upon the magnitude and direction of electrically-driven flow, i.e., electroosmosis, or various other sources of flow such as pressure, convection, capillarity, etc. Voltage gradients may likewise be manipulated to provide electrophoretic migration in either direction.

The introduction of an electric field resulting in electrophoretic migration of a specific sample component, coupled with manipulation of bulk flow equal and opposite to electrophoretic migration, results in cessation of movement of those specific sample components. Thus, the independent control of these parameters provides for absolute control of movement of sample components within the fluid about a micro-device.

The method of the invention comprises as a first step, the introduction of a sample containing the sample component of interest into a channel or capillary that has been filled with buffer. Sample introduction may be accomplished using a syringe by which the sample solution is injected into the channel. Alternatively, the introduction of the sample can be performed according to standard procedures, including but not limited to the use of electroosmotic flow, electro-kinetic pumping, or pneumatic pumping.

An electrophoretic arrangement in which a capillary is utilized to create the restricted flow path is shown in Figure 1. In this arrangement electrodes 20 are located external to and mounted onto a fused silica capillary. A counter electrode 24 is placed at a location remote from electrodes 20 and forms a circuit therewith. A

high voltage is applied to the electrodes 20 and 24 by power supply 26. A reservoir 28 including buffer bulk flow materials is in fluid contact with the capillary. A sample 5 including charged components is introduced into the reservoir and moves towards the entrance 9 of the capillary in the presence of the applied voltage which induces electrophoretic migration. Thus, the charged components in analyte 5 are concentrated at the entrance 9 of the capillary 22.

The present invention also provides a micro-analytical separation device comprised of etched or molded channels whereby various channels are used for separation and analysis purposes and others are distinctly used for the purpose of injection or material movement illustrated in Figs. 2(a-c). As shown in Figure 2a the system includes an injection channel 2 and separation channel 4 . Sample material is injected to fill the injection channel 2 in between the separation channels 4 as depicted in Figure 2b. To prevent unintentional introduction of material movement, commonly referred to as trailing or leaking, into the main separation channel after injection ceases, a small voltage is applied to the two injection channel electrodes 5. As illustrated in Figure 2c, after the initial injection the electrodes are used to create an appropriate voltage gradient to prevent unwanted introduction of materials into the separation channel thereby concentrating desired components in separation channel 4. By manipulating flow and the voltage fields independently, positive, negative and neutral molecules may be manipulated as a group or individually.

A high voltage is applied by power supply means between the inlet and outlet end of the channel or capillary through electrode means. The voltage used is not critical to the invention and may vary widely depending on the sample component(s) to be excluded or concentrated. Conditions for selecting appropriate voltage conditions will depend on the physical properties of the sample component(s) and can be determined by those of skill in the art.

To preconcentrate sample components of either positive or negative charge, the method of the invention further comprises setting the bulk flow in the channel or capillary approximately equal to and opposite to the electrophoretic migration rate of the material. The bulk flow in the capillary may be generated and controlled by either electroosmosis, pressure or various other mechanisms. Bulk flow

may be created and controlled by electroosmotic pumping devices, pneumatic devices, or directly by electroosmosis with dynamic control and monitoring. Thus the sample component of interest is drawn toward the channel by bulk flow, but is excluded from the channel by the voltage field effects on a narrow range of materials with similar electrophoretic migration rates thereby excluding or concentrating the sample component of interest at the immediate entrance of the capillary or channel.

Alternatively, any constrained fluid pathway, for example a fused silica or teflon capillary, where separation or injection of materials of interest are performed may be included in the device. Each channel or continuous fluid pathway where control of material movement is desired is constructed with an electrode adjoining the entrance and exit of the channel or pathway. Electrodes are placed at the entrance of the side channels to control the voltage field allowing electrophoretic migration to occur, and electroosmosis if the source of flow in the particular channel. In this manner the invention provides for integration of preconcentration and analysis within the constrained fluid pathway.

In the preferred embodiment of the invention as shown in Figure 2a-c, the injection channel 2 is perpendicular to the separation channel 4, although the geometry of this intersection is not of direct importance to the concepts presented here. Electrodes 5, 6 are located at the immediate entrances of channels 5, 6 and are electrically connected to the junction where the two channels 2, 4 intersect. Placement of an electrode at the immediate entrance of a capillary or channel and at the junction with another channel or buffer reservoir, creates a chemical voltage gate, in that movement of materials may be independently controlled by simply varying the voltage field gradient and the flow rate within the particular channel. At this chemical voltage gate, materials of interest may be totally excluded from entering the adjoining channel or selectively permitted to enter the channel by using electrophoretic focusing techniques.

In another embodiment of the invention shown in Figure 3 a reservoir containing a buffer solution 5 is placed in fluid contact with a channel 12 and an electrode 9 is placed at the immediate entrance to that channel 11. The buffer reservoir is maintained at the same voltage as the entrance electrode, thus the material

will not undergo electrophoretic migration within the reservoir. However, the charged materials will move toward the channel entrance at the same rate as the bulk flow. At the immediate entrance of the channel the effects of the applied voltage field influences the charged materials, thus inducing electrophoretic migration. Since the bulk flow within the channel is approximately equal to and opposite the electrophoretic migration, the charged material of interest stops.

The flow rate of fluids may be controlled by, for example, the following techniques: pressure induced flow, capillary, and electroosmosis as taught by Giddings (1991, Unified Separations Science, Wiley-Interscience, New York, Chapt. 3). More specifically, pressure can be controlled by any physical or chemical means which will generate controllable flow or pressure. Capillarity can be controlled via chemical, electrochemical or photo-induced surface or solution changes as taught by Gallardo et al. (1999, Science 283:57-60). Electroosmosis can be controlled by external radial electrostatic fields as taught by Tsuda (1998, Handbook of Capillary Electrophoresis, Ed. J.P. Landers, 2nd ed., CRC Press, Boca Raton , Chap. 22).

The methods and devices of the present invention may be used for purposes of manipulating, testing, probing, or analyzing fluids of any kind where fluid manipulations may be used for preconcentration, chemical reaction, injection, detection, or movement or restriction of movement, of the materials of interest. The manipulations provided for by the methods and devices described herein will allow for precise liquid injection and handling within a micro-chemical analysis device in addition to the ability to increase local concentration of materials by several orders of magnitude.

Preparation of specific embodiments in accordance with the present invention will now be described in further detail. These examples are intended to be illustrative and the invention is not limited to the specific materials and methods set forth in these embodiments.

The examples discussed hereinafter were conducted using the following standard chemicals and instrumentation, unless otherwise stated:

Chemicals and Materials. Sodium dihydrogen phosphate and anhydrous ethyl alcohol (Aldrich Chemical Company, Milwaukee, WI); and

phosphoric acid (EMG/NCV Science, Gibbstown, NEW JERSEY) were used as received. Capillaries were 45 cm in length (150 μm o.d. - 20 μm i.d.) fused silica and were purchased from Polymicro Technologies (Phoenix, AZ). 0.2 μm carboxylate modified yellow-green fluorescent (505/515) latex micro spheres were purchased from Molecular Probes (Eugene, OR). The capillary electrophoresis buffer used for the latex micro sphere experiments was 100 mM phosphate buffer, adjusted with phosphoric acid to pH 5.1.

Instrumentation. The capillary electrophoresis system was built and used a CZE1000R high voltage power supply from Spellman High Voltage Electronics Corporation (Hauppauge, New York). The vacuum pump system was purchased from Cenco Hyvac (Fort Wayne, IN). The laser source was a 442/325 nm 100 MPA: (Omnichrome Laser, Chino, Cat Scan). Image viewing was accomplished with a case closed-5E CCD camera (HutchNet, East Hartford, Construction) integrated to an Olympus Vanex stereo microscope (Tokyo, Japan). Data collection and analysis were accomplished using Labview software and an Imaq Pci- 1408 image acquisition board by in-house program development (National Instruments, Austin, TX). Data analysis was also performed on Microsoft Excel spreadsheet program using an Optiplex GXI Pentium 233 (Dell Computer Corporation, Round Rock, TX). The fluorescent signal was monitored from the carboxylate modified latex micro spheres as vacuum and voltage fields were adjusted.

Example 1

Experiments were performed to effectively demonstrate the increased local concentration of specific materials using a capillary 30 and reservoir 32 arrangement shown in Fig. 4. The tip of the capillary was coated with metal 34 thereby providing a metal electrode. These experiments were performed with fluorescence microscopy, fluorescently labeled latex microspheres, vacuum flow and a metal-coated capillary tip.

The presence or location of carboxylate-modified latex spheres were directly observed with the microscope under the effects of vacuum induced flow. The voltage was then empirically adjusted until the micro spheres were excluded from entering the capillary due to the electrophoretic migration rate of the micro spheres.

The intensity of the fluorescent signal which is directly related to concentration was monitored. Only a selected probe area, of approximately 2.5 μm x 120 μm parallel, and centered with the bore of the capillary immediately outside the entrance was quantitated for the fluorescence intensity changes.

First, control experiments were performed to determine if adsorption or other unknown processes were responsible for the increased fluorescence. These control experiments consisted of using either the voltage field only (- 14 kV) or the vacuum-induced flow only 1.2 in Hg across a 45 cm long, 20 μm i.d. capillary. As illustrated in Figure. 5 the fluorescent signal was monitored and quantitated for 4 minutes. The fluorescent signal was normalized with the fluorescent signal obtained at t = 0 minutes to eliminate any existing background fluorescence from the temporal data. The normalized fluorescent signal of the control experiments remained at a value of 1.75±9.32 (n = 11) throughout the 4 minutes of the experiment (Figure 6). No increase in fluorescent intensity was observed over the experimental period indicating that no unknown mechanisms nor adsorption to the capillary tip and walls contributed to the increased fluorescent intensity in the following experiments.

Experiments were performed to demonstrate preconcentration once the electrophoretic migration rate within the channel in the capillary was adjusted to be equal to and opposite the bulk buffer low rate. As with the control experiments, the fluorescence intensity was normalized and then monitored for 4 minutes (n = 4). The voltage empirically determined to generate an electrophoretic migration rate which counterbalanced the bulk flow rate was 14kV. Figures 7(a) and (b) are fluorescence micrographs of a capillary entrance before and after, respectively, preconcentration of 200 nm fluorescently labeled latex micro spheres for 270 seconds. As illustrated in Figure 7b and Figure 4 the largest fluorescence intensity changes occurred within 33 μm of the capillary entrance. Due to dynamic range limitations, the fluorescent intensity at the entrance to the capillary saturated the CCD and therefore quantitation of this effect must be performed some 19.2 μm outside the entrance to the capillary. The normalized fluorescent signal at 19.2 μm resulted in an increase in fluorescence intensity approximated by a linear equation ($y = mx + b$) where m is 0.042 arb. units/min and b is 0.99 arb. units ($R^2 = 0.938$, $P \leq 0.01$). The initial concentration of

the micro-spheres was 1. 473 x 10^{10} micro spheres/mL.

The preconcentration build-up over time can be modeled as the formation as an exponential zone superimposed on a background of constant solute concentration for materials accumulating up behind a partially rejecting barrier such a filter. The filter in this case is the exclusion of the micro spheres from the capillary by the applied voltage field and the resulting electrophoretic migration rate. Assuming the system will reach steady state conditions after a given time, the background concentration of the micro spheres is equal to J_α/v , initial flux over velocity. The concentration build-up of the micro spheres is given by the following equation:

$$c = J_\alpha v + (c_o - J_\alpha 1\ v)\exp(-|v|y/D_\gamma)$$

where the concentration of the micro spheres is given by c, the flux of the micro spheres is given by J_α, the velocity of the flow towards the barrier is v, the original concentration of micro spheres is given by c_o, the distance from the barrier is given by y, and the total diffusion of the micro spheres is given by D_γ. A plot of concentration of desired components versus location in the arrangement is shown in Figure 4.

Although the present invention has been described with reference to latex micro spheres and fused silica capillaries providing the constrained fluid pathway, it should be understood that various modifications and variations can be easily made by those skilled in the art without departing from the spirit of the invention. Such modifications are intended to fall within the scope of the claims. Accordingly, the foregoing disclosure should be interpreted as illustrative only and not in a limiting sense. Various publications are cited herein, the contents of which are incorporated, by reference, in their entireties.

CLAIMS

1. A method for controlling the movement of a specific sample component in a fluid sample comprising:

   (a) providing a constrained fluid pathway having an inlet;

   (b) introducing the fluid sample into the inlet of the constrained fluid pathway;

   (c) providing an electrode mounted at the inlet of the fluid pathway, the electrode being entirely external to the constrained fluid pathway;

   (d) applying voltage to the electrode to create a voltage gradient within the constrained fluid pathway to promote electrophoretic migration of the sample component; and

   (e) adjusting the flow rate of the fluid approximately equal to and opposite to the electrophoretic migration;

   wherein movement of the specific sample component ceases.

2. The method of claim 1 wherein the constrained fluid pathway is a channel.

3. The method of claim 1 wherein the constrained fluid pathway is a capillary tube.

4. The method of claim 1 wherein the constrained fluid pathway is less than 200 microns in diameter.

5. The method of claim 1 wherein the flow rate of the fluid sample is controlled by electroosmosis.

6. The method of claim 1 wherein the flow rate of the fluid sample is controlled by pressure.

7. The method of claim 1 wherein the constrained fluid pathway is a channel on a microchip.

8. An electrophoretic apparatus for controlling the movement of an sample component in a fluid sample comprising:

(a) at least one constrained fluid pathway having an inlet and an electrode mounted at the inlet of the constrained fluid pathway and entirely external to the constrained fluid pathway; and

(b) a power supply for supplying a voltage to the electrode.

9. The apparatus of claim 8 wherein the constrained fluid pathway is a channel located on a microchip.

10. The apparatus of claim 8 wherein the constrained fluid pathway is a capillary.

11. The apparatus of claim 8 further comprising a buffer reservoir for containing a buffer solution in fluid contact with the constrained fluid pathway.

12. The apparatus of claim 8 wherein the constrained fluid pathway is a channel in a microchip.

13. The apparatus of claim 8 wherein the constrained fluid pathway is a capillary.

14. The apparatus of claim 8 wherein the diameter of the constrained fluid pathway is less than 200 microns in diameter.

15. An electrophoretic apparatus for controlling the movement of an sample component in a fluid sample comprising:

(a) at least one injection fluid pathway having an electrode mounted at the inlet of said the pathway;

(b) at least one separation or further fluid transfer fluid pathway having an electrode mounted at the inlet of said pathway;

(c) at least one power supply for providing voltage to the electrodes; and

(d) means for regulating the bulk flow within the channels.

16. The method of claim 15 wherein the constrained fluid pathway is a channel in a microchip.

17. The method of claim 15 wherein the constrained fluid pathway is a capillary.

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(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
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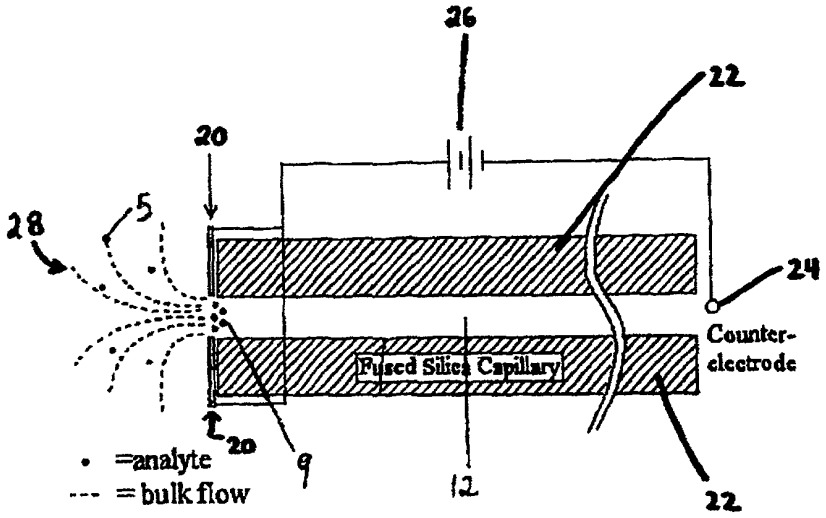
(54) Title: CONTROL OF FLOW AND MATERIALS FOR MICRO DEVICES



(57) Abstract

The present invention generally relates to methods and devices for the control of the movement of fluids and electrically charged sample components within those fluids. More particularly, the present invention permits exclusion or concentration of specifically chosen sample components within a fluid. The present invention provides an analytical device, either microchip– or capillary–based, having the means to exclude specific sample components of interest from a capillary or channel for the purpose of preconcentration or control of movement of sample components. Such a control system includes a means for controlling the flow of the fluid in the channel and the placement of an electrode at the immediate entrance of each channel on such devices so that material may be directly manipulated by effects of both bulk flow and electrically driven migration.

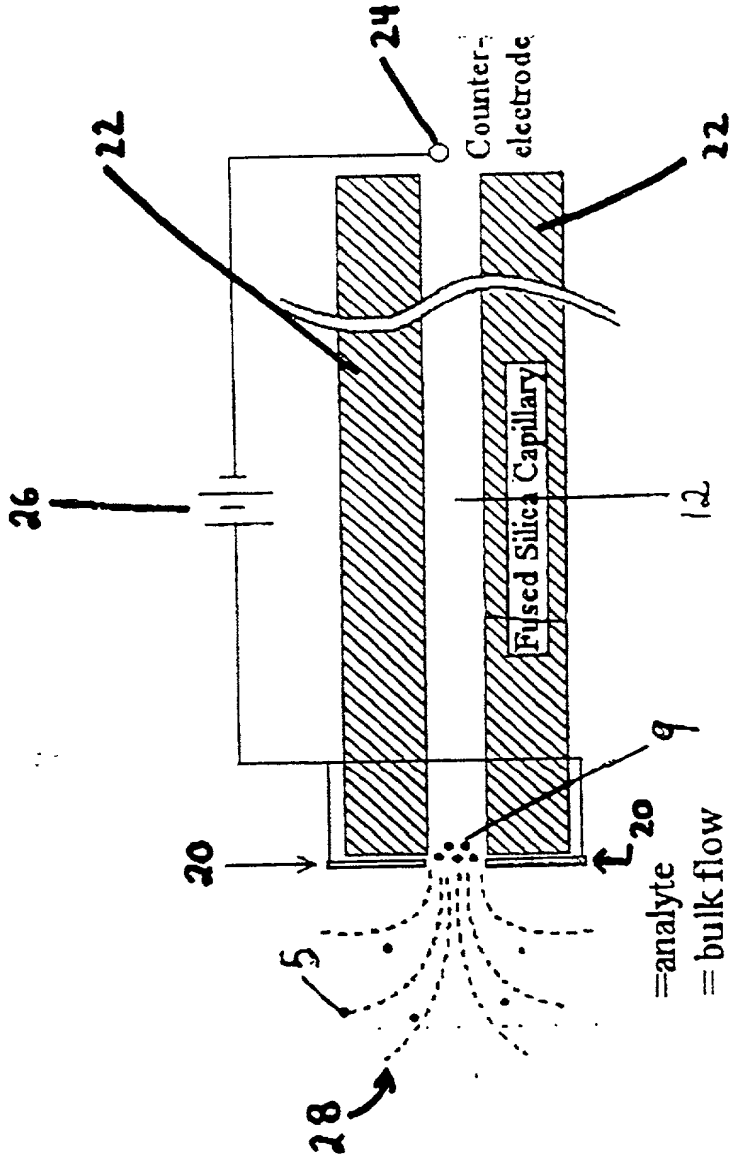

24
Counter-electrode
22
22
26
Fused Silica Capillary
12
9
20
20
5
18
=analyte
=bulk flow

Fig. 1

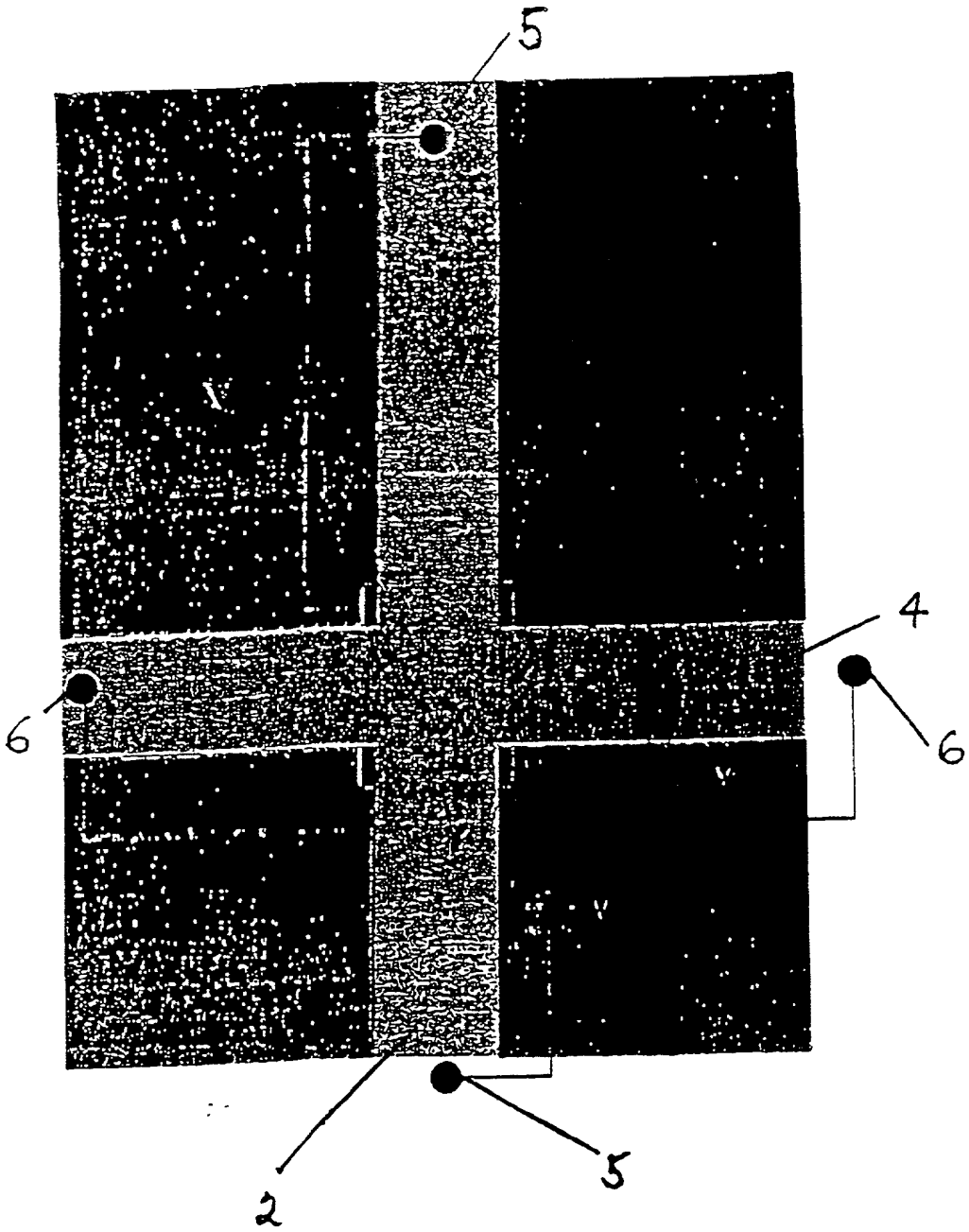


Figure 2a

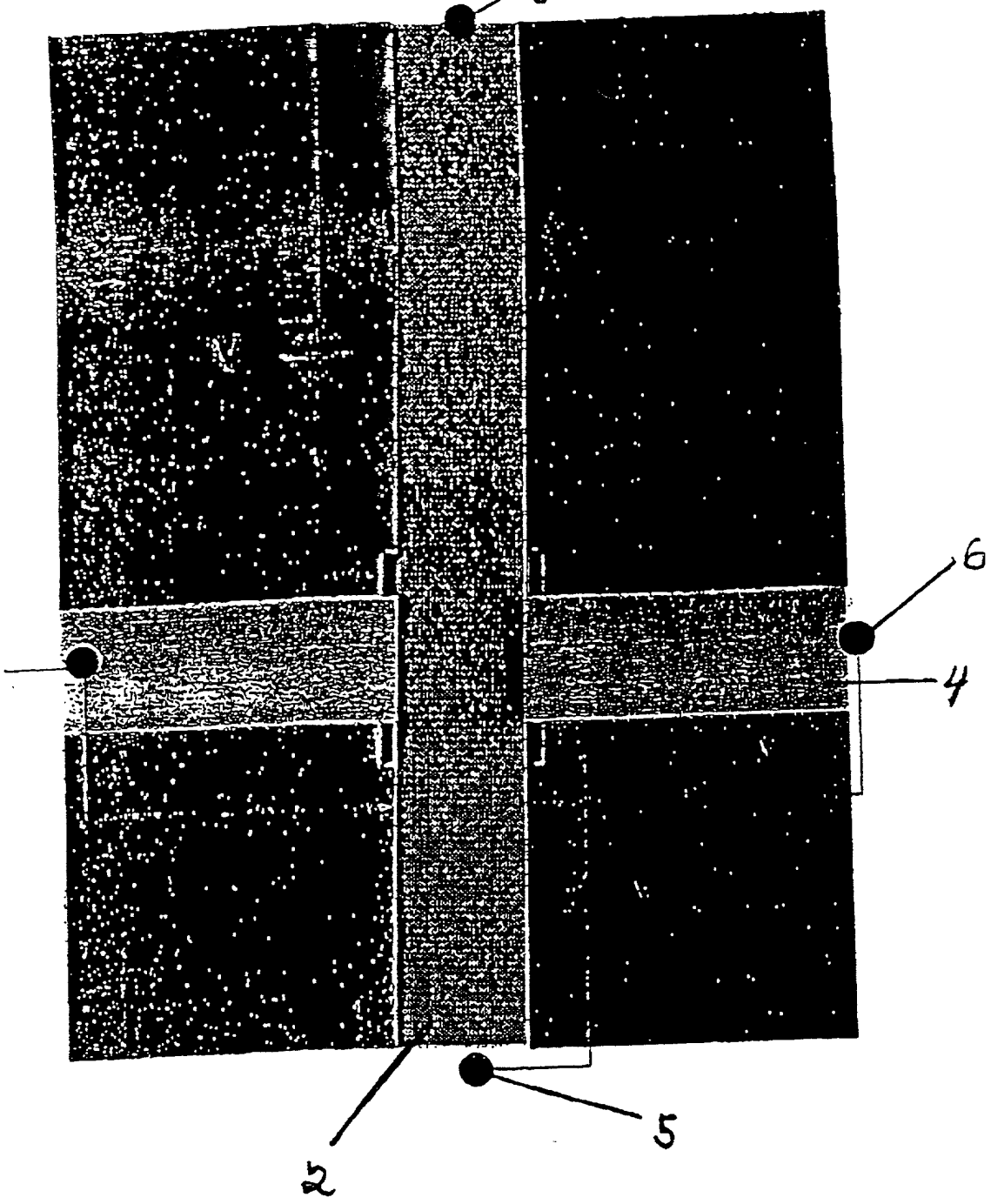


Figure 2b

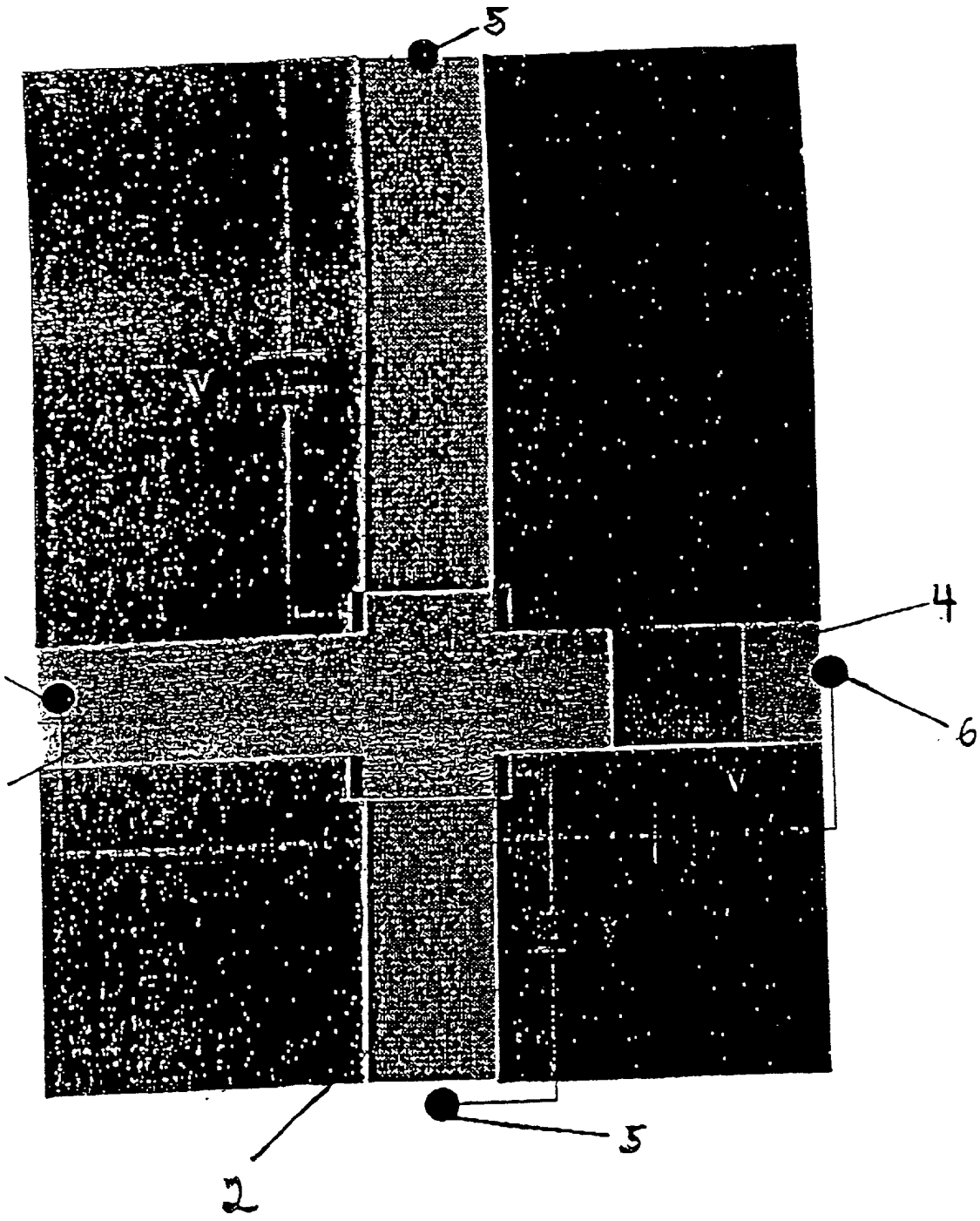


Figure 2c

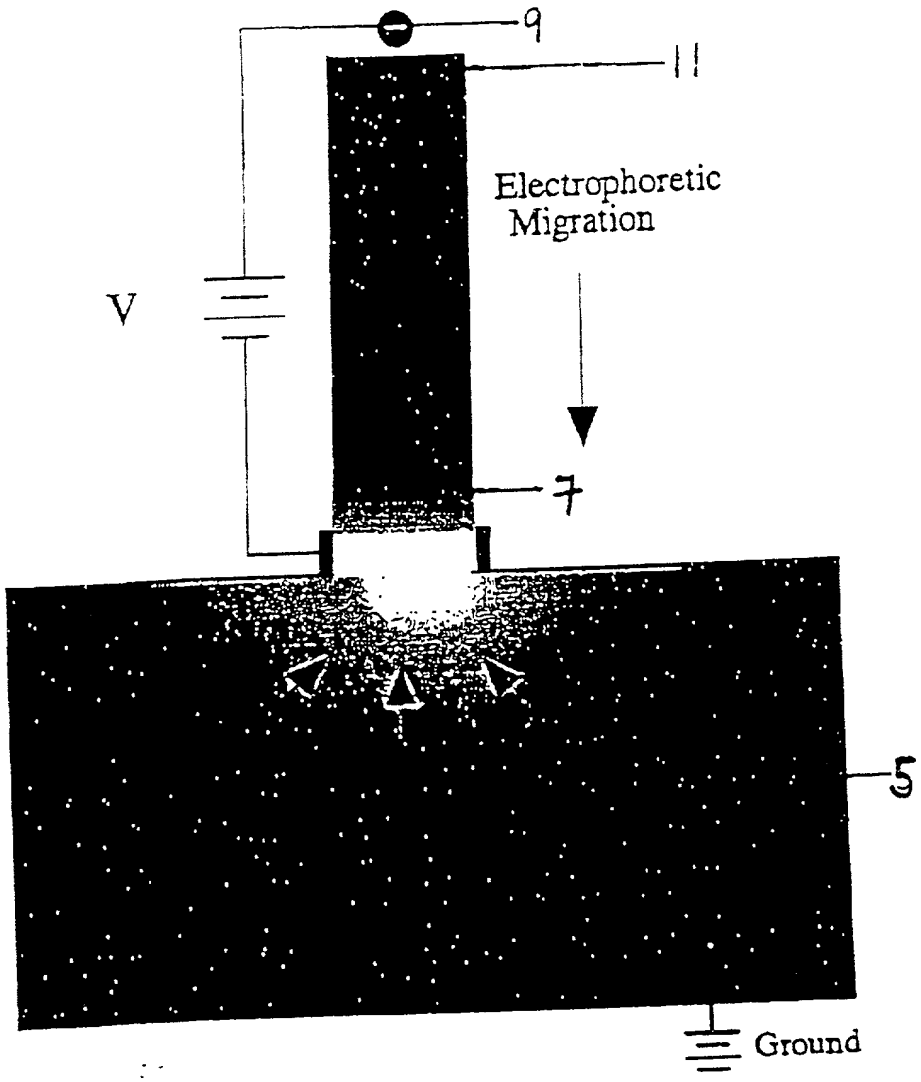


Fig. 3

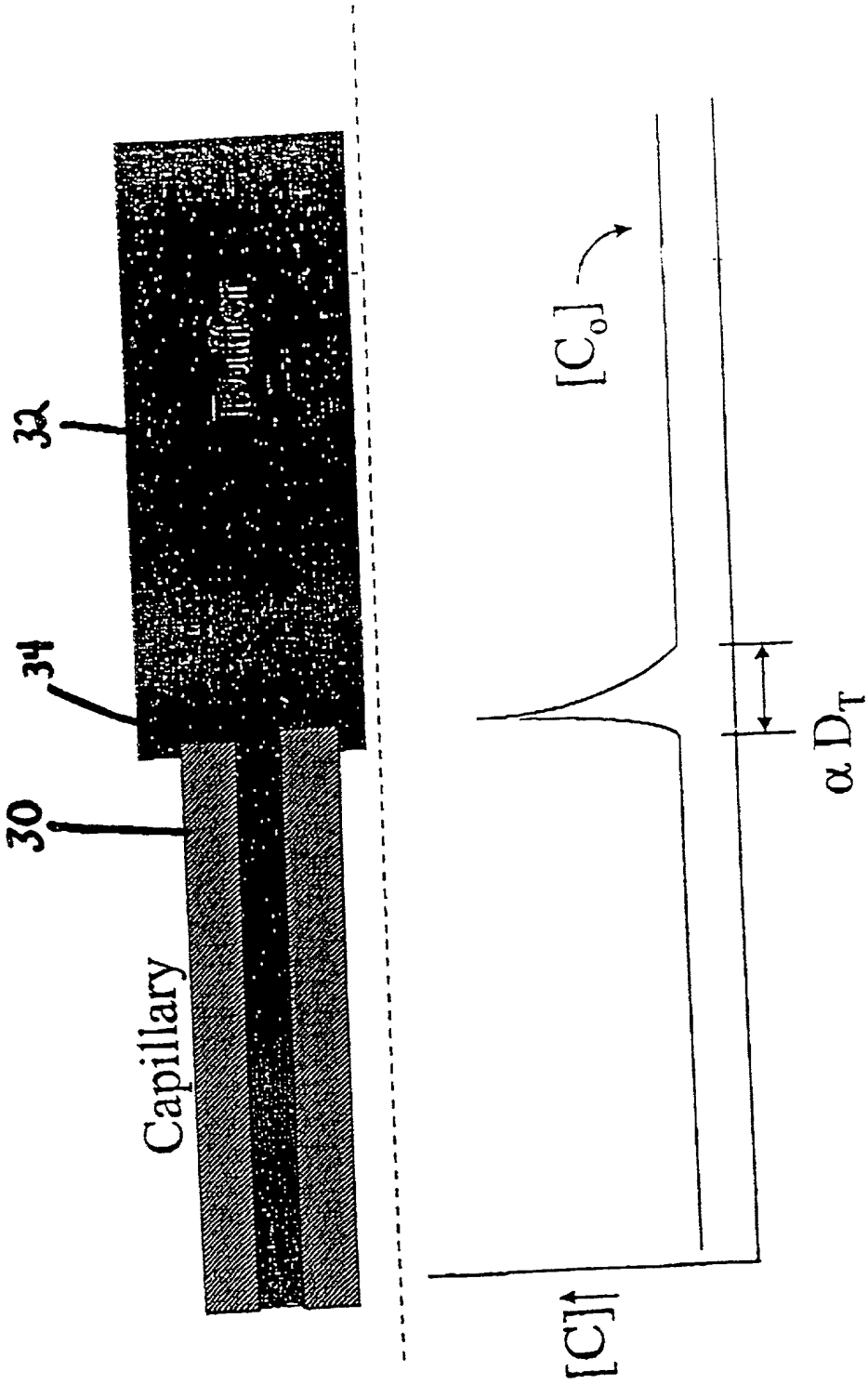


Fig. 4

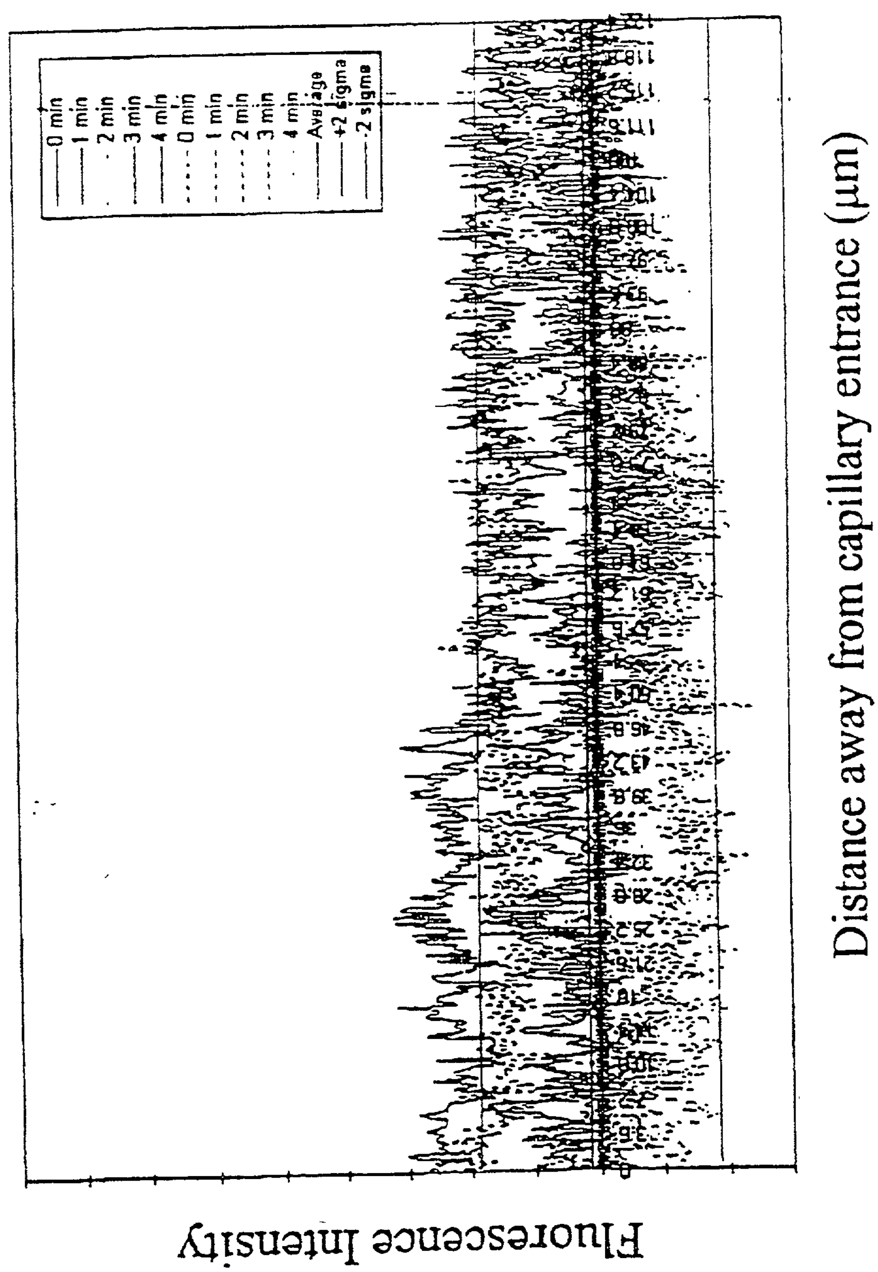

0 min
1 min
2 min
3 min
4 min
0 min
1 min
2 min
3 min
4 min
Average
+2 sigma
-2 sigma
Distance away from capillary entrance (μm)
Fluorescence Intensity

Fig. 5

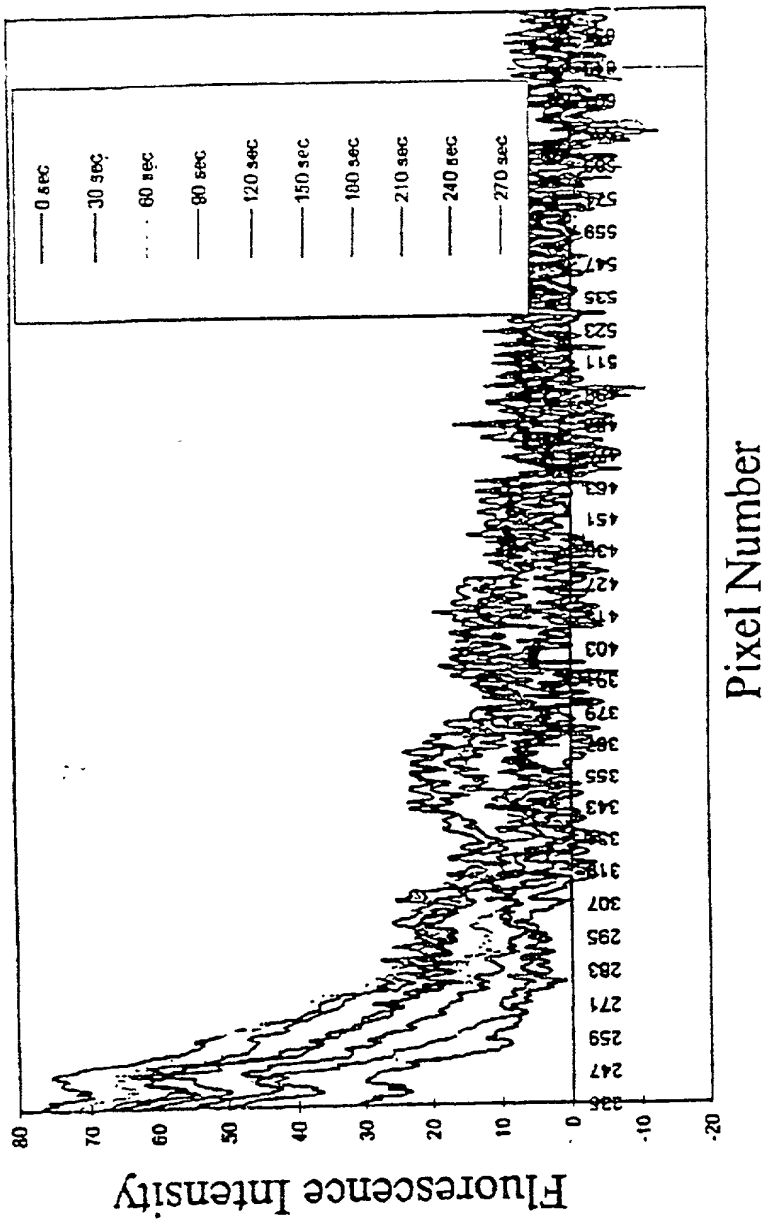

0 sec
30 sec
60 sec
90 sec
120 sec
150 sec
180 sec
210 sec
240 sec
270 sec
Fluorescence Intensity
Pixel Number

Fig. 6

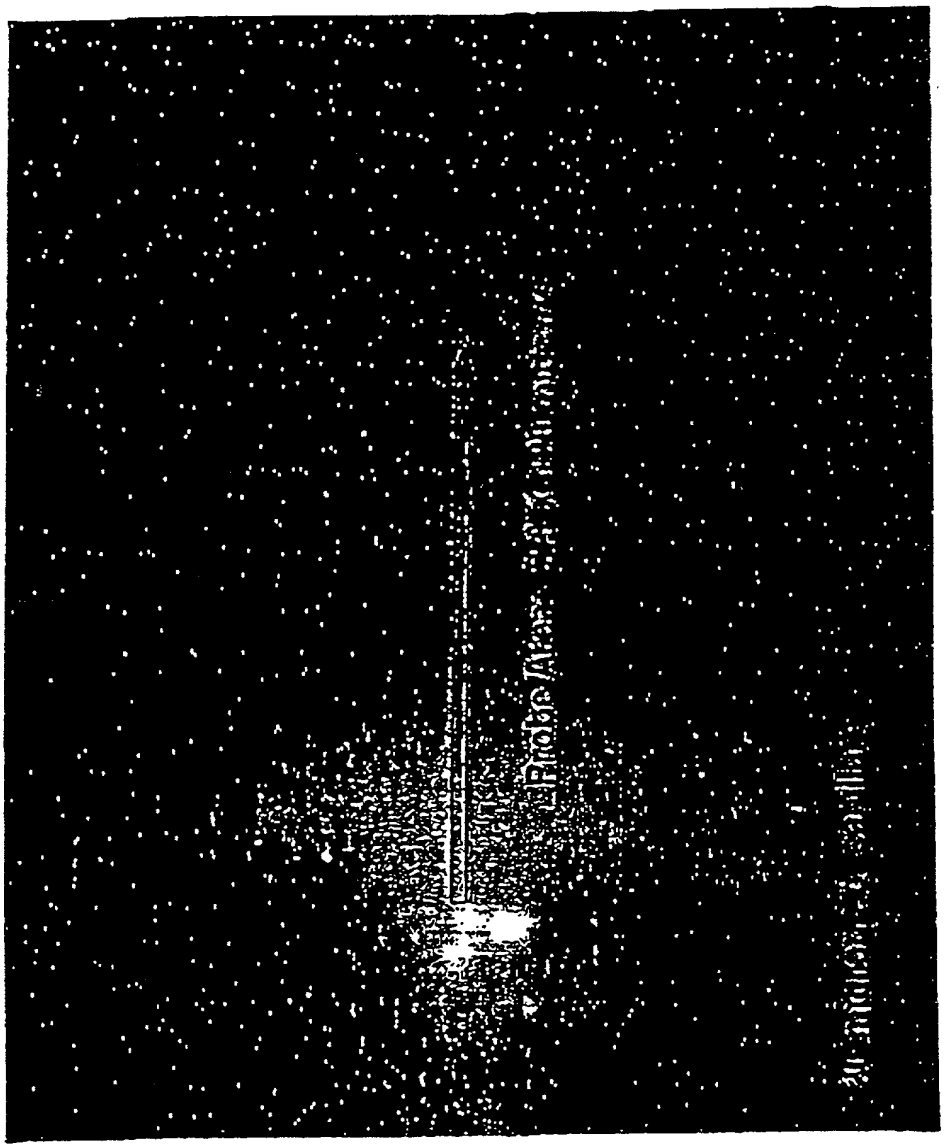


Fig. 7a

4 minute focus, -14 kV, 30.5 mm Hg, 40.5 cm tube

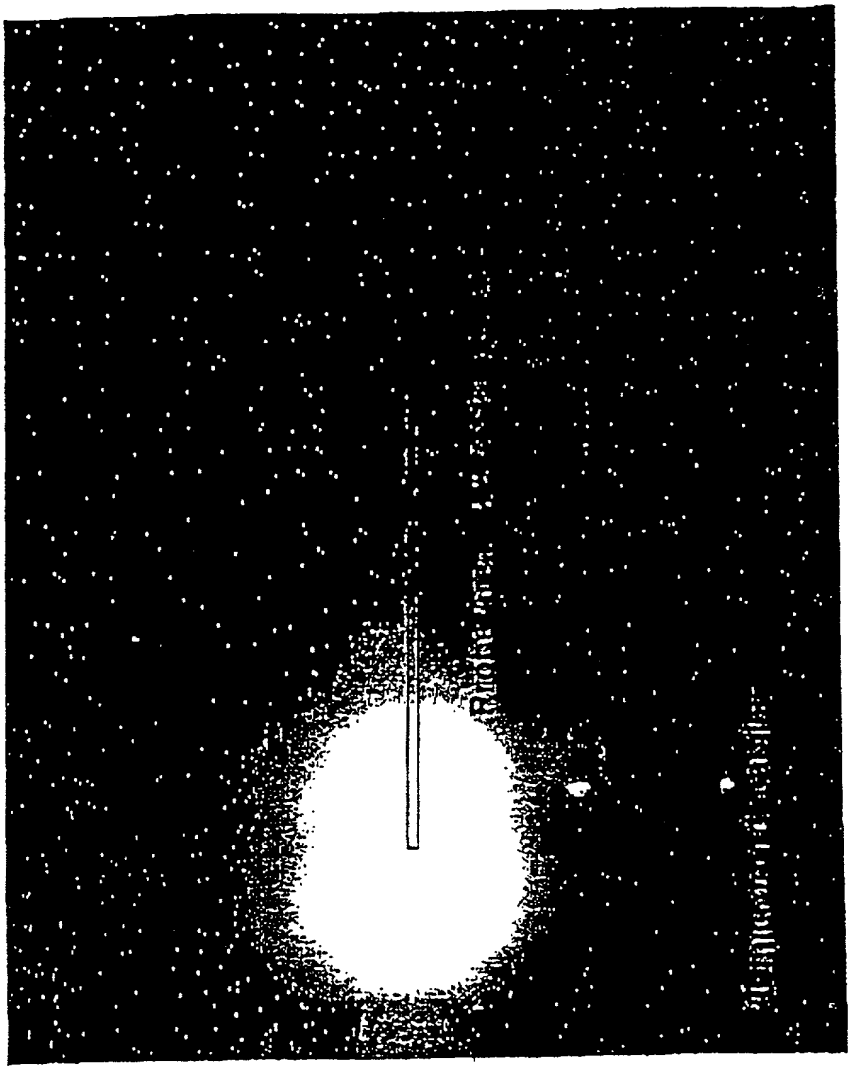


Fig. 7b

COMBINED DECLARATION AND POWER OF ATTORNEY

(Original, Design, National Stage of PCT, Divisional, Continuation or C-I-P Application)

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CONTROL OF FLOW AND MATERIALS FOR MICRO DEVICES

This declaration is of the following type:

[] original
[] design
[X] national stage of PCT.
[] divisional
[] continuation
[] continuation-in-part (C-I-P)

the specification of which: *(complete (a), (b), or (c))*

(a) [X] is attached hereto.
(b) [ ] was filed on as Application Serial No. and was amended on *(if applicable).*
(c) [X]was described and claimed in PCT International Application No. PCT/US99/13340 filed on July 29, 1999 and was amended on *(if applicable).*

Acknowledgement of Review of Papers and Duty of Candor

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56.

[ ] In compliance with this duty there is attached an information disclosure statement. 37 CFR 1.98.

Priority Claim

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International Application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International Application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application on which priority is claimed

(complete (d) or (e))

(d) [X] no such applications have been filed.
(e) [ ] such applications have been filed as follows:

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
COUNTRY APPLICATION NO.	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
			[ ] YES NO [ ]
			[ ] YES NO [ ]
			[ ] YES NO [ ]
ALL FOREIGN APPLICATION[S], IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION			
			[ ] YES NO [ ]
			[ ] YES NO [ ]
			[ ] YES NO [ ]

Claim for Benefit of Prior U.S. Provisional Application(s)

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Provisional Application Number	Filing Date
60/088,956	June 11, 1998

Claim for Benefit of Earlier U.S./PCT Application(s) under 35 U.S.C. 120

(complete this part only if this is a divisional, continuation or C-I-P application)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

Power of Attorney

As a named inventor, I hereby appoint Dana M. Raymond, Reg. No. 18,540; Frederick C. Carver, Reg. No. 17,021; Francis J. Hone, Reg. No. 18,662; Joseph D. Garon, Reg. No. 20,420; Arthur S. Tenser, Reg. No. 18,839; Ronald B. Hildreth, Reg. No. 19,498; Thomas R. Nesbitt, Jr., Reg. No. 22,075; Robert Neuner, Reg. No. 24,316; Richard G. Berkley, Reg. No. 25,465; Richard S. Clark, Reg. No. 26,154; Bradley B. Geist, Reg. No. 27,551; James J. Maune, Reg. No. 26,946; John D. Murnane, Reg. No. 29,836; Henry Tang, Reg. No. 29,705; Robert C. Scheinfeld, Reg. No. 31,300; John A. Fogarty, Jr., Reg. No. 22,348; Louis S. Sorell, Reg. No. 32,439; Rochelle K. Seide Reg. No. 32,300; Gary M. Butter, Reg. No. 33,841; Marta E. Delsignore, Reg. No. 32,689; and Lisa B. Kole, Reg. No. 35,225 of the firm of BAKER BOTTS L.L.P., with offices at 30 Rockefeller Plaza, New York, New York 10112, as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith

SEND CORRESPONDENCE TO:	DIRECT TELEPHONE CALLS TO:
BAKER BOTTS L.L.P. 30 ROCKEFELLER PLAZA, NEW YORK, N.Y. 10112 CUSTOMER NUMBER: 21003	BAKER BOTTS L.L.P. (212) 705-5000

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00 FULL NAME OF SOLE OR FIRST INVENTOR	LAST NAME HAYES	FIRST NAME MARK	MIDDLE NAME A.	
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POST OFFICE ADDRESS	POST OFFICE ADDRESS 3583 W. Barcelona Drive	CITY Chandler	STATE or COUNTRY Arizona	ZIP CODE 85226
DATE 11-21-00	SIGNATURE OF INVENTOR Mark A. Hayes			
2-00 FULL NAME OF SECOND JOINT INVENTOR, IF ANY	LAST NAME POLSON	FIRST NAME NOLAN	MIDDLE NAME A.	
RESIDENCE & CITIZENSHIP	CITY Chandler	STATE or FOREIGN COUNTRY Arizona	COUNTRY OF CITIZENSHIP United States USX	
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DATE Nov. 21, 2000	SIGNATURE OF INVENTOR			
FULL NAME OF THIRD JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE or COUNTRY	ZIP CODE
DATE	SIGNATURE OF INVENTOR			
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE or COUNTRY	ZIP CODE
DATE	SIGNATURE OF INVENTOR			

Check proper box(es) for any added page(s) forming a part of this declaration

[ ] Signature for ninth and subsequent joint inventors. Number of pages added _____.

[ ] Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. Number of pages added _____.

[ ] Signature for inventor who refuses to sign, or cannot be reached, by person authorized under 37 CFR 1.47. Number of pages added _____.